



Europäisches Patentamt
European Patent Office
Office européen des brevets

⑪ Publication number:

0 171 148
A1

B42

⑫

EUROPEAN PATENT APPLICATION

⑬ Application number: 85304189.7

⑮ Int. Cl. 4: G 01 N 33/543, G 01 N 21/77

⑭ Date of filing: 12.06.85

⑯ Priority: 13.06.84 GB 8415018
13.06.84 GB 8415019

⑰ Applicant: UNILEVER PLC, Unilever House Blackfriars
P.O. Box 68, London EC4P 4BQ (GB)
⑱ Designated Contracting States: GB

⑲ Date of publication of application: 12.02.86
Bulletin 86/7

⑳ Applicant: UNILEVER NV, Burgemeester
s'Jacobplein 1 P.O. Box 780, NL-3000 DK Rotterdam (NL)
㉑ Designated Contracting States: BE CH DE FR IT LI NL
SE AT

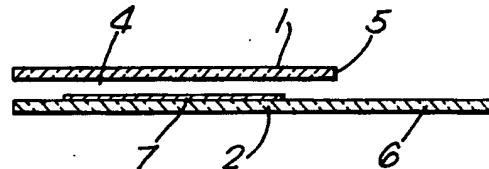
㉒ Designated Contracting States: AT BE CH DE FR GB IT
LI NL SE

㉓ Inventor: Shanks, Ian Alexander, 56 The Bury, Pavenham
Bedford MK43 7TY (GB)
Inventor: Smith, Alan Martin, 23 The Marsh, Carlton
Bedford MK43 7JU (GB)

㉔ Representative: Stansliffe, Terence Christopher et al,
UNILEVER PLC Patents Division P.O. Box 68 Unilever
House, London EC4P 4BQ (GB)

㉕ Devices for use in chemical test procedures.

㉖ A specifically-reactive sample-collecting and testing device
possessing a cavity or cavities each having a dimension small
enough to enable sample liquid to be drawn into the cavity by
capillary action, wherein a surface of the cavity carries an
immobilised reagent appropriate to the test to be carried out in the
device, and wherein said surface is a surface of a transparent
solid plate to act as a light-transmissive waveguide and forming a
wall of the cavity, said plate having an edge which is substantially
optically smooth and transverse to the plane of the plate.



EP 0 171 148 A1

- 1 -

P3003 AA
ver: 7 Jun 85

Devices for Use in Chemical Test Procedures

This invention relates to devices for use in chemical (especially biochemical or clinical) test procedures, to processes for their manufacture, and to the uses of the devices.

The devices are, in certain embodiments, intended for use in specific binding assay procedures, among which an important group is constituted by immunoassay procedures. Examples of such immuno assays, especially enzyme-linked immunoassays, are cited in Specifications Nos. EP 0 042 755, GB 2 074 727, GB 2 086 041, and GB 1 548 741.

Previously, immunoassay procedures have often been carried out using so-called microtitre wells, conventionally of about 0.5ml working capacity, amongst a variety of other liquid containers for the assay reaction liquids. Other devices and arrangements for handling immunoassay materials are described in Specifications Nos. EP 0 31 993, GB 1 571 872, GB 1 584 129 and GB 1 414 479, for example.

In particular, the prior art contains numerous disclosures of analytical devices for handling and metering small volumes of test samples.

- 5 GB 2 090 659 (Instrumentation Laboratory, Inc.) describes test strips constructed with a self-filling metering channel and a lip or inlet on which a sample of more than about 10 microlitres of for example whole blood can be placed, so that (for example) 10 microlitres is
- 10 taken up by capillary action to react with a reagent carried on a fibrous pad above a filter layer beneath a transparent window. The result can be viewed by the unaided eye, e.g. as a colour reaction.
- 15 GB 2 036 075 (H E Mennier), GB 1 104 774 (J P Gallagher), EP 0 057 110, 0 034 049, 0 010 456 (Kodak), all describe some other aspect of the uses of capillary channel or chamber dimensions for handling biological or test fluids.
- 20 GB 1 530 997 (Monsanto) describes the use of coated optical fibres which can be used in tests that change the light transmitting capabilities of the waveguides via reactions of the coatings, e.g. antigen-antibody
- 25 reactions. WO 81/00912 (Buckles) also describes fibre-optic devices in which the fibre surface or surroundings modify the light transmission through the core.
- 30 USP 3 939 350 describes optical measurement of fluorescent material bound to the surface of a solid transparent prism by a method involving a single total internal reflection and interaction of the evanescent wave at the surface of the prism with the bound material.

EP 0 075 353 (Battelle) makes specific reference to the exponentially-decaying (evanescent) external radiation due to light which is propagated longitudinally in a fibre, and its interaction with coatings, and this 5 principle is also taken up in the immunoassay test devices of EP 0 103 426 (Block) in which light of fluorescence excitation as well as emission wavelengths is propagated within a antigen - or antibody - coated optical fibre or plate contacting a capillary-dimensional sample liquid 10 volume bounded by a tube or another plate and containing a fluorescent-tagged binding partner of the material coated on the fibre or plate.

According to the invention to be described here, 15 capillary fill cell devices which can be conveniently manufactured, are provided to facilitate in particular specific binding assays using very small liquid samples.

According to the invention we provide a specifically-reactive sample-collecting and testing device possessing a 20 cavity or cavities each having a dimension small enough to enable sample liquid to be drawn into the cavity by capillary action, wherein a surface of the cavity carries an immobilised reagent appropriate to the test to be 25 carried out in the device, and wherein said surface is a surface of a transparent solid plate to act as a light-transmissive waveguide and forming a wall of the cavity, said plate having an edge which is substantially optically smooth and transverse, e.g. at some transverse angle but 30 most preferably perpendicular, to the plane of the plate.

The devices enable convenient sample collection and carrying out optical analysis in situ of the products of reaction of the sample with reagent(s) contained in the 35 devices. The waveguide plate can be transparent to infrared, visible, and/or ultraviolet light, and one method

of using the devices is to arrange for a fluorescent material to become bound to the immobilised reagent to a variable extent depending on the assay and the sample material of interest, and then to carry out optical measurement of the resulting bound fluorescent material.

The immobilised reagent in the device can for example be an immobilised antigen or antibody which is capable of binding a fluorescent or luminescent or coloured component 10 of an assay mixture. But it is understood that any immobilised material can be used that specifically interacts with another component of a test reaction material in a way that gives optically measurable results, depending only on the kind of test in question.

15 The surface of the cavity opposite to the reagent-bearing waveguide surface can, as in examples illustrated below, be formed of a second similar transparent plate. However, this is not necessary, and 20 for some forms of test it may be suitable to use a light-absorbing or opaque or reflective opposite wall to the capillary cavity.

In some useful examples of the devices of this 25 invention, a selective barrier such as a filter or dialysis membrane can be fitted to ensure that the takeup of sample liquid into the capillary-dimension cavity is selective to exclude unwanted material. Such unwanted material will depend on the nature of the test but may 30 include cells such as blood cells, cell debris or dispersed high-molecular-weight material. A suitable filter can be for example a paper filter located at the sample entrance to the capillary cavity or a strip or area 35 of filter or dialysis membrane material fixed at or in the capillary cavity.

It is also within the scope of the invention that the area covered by an immobilised reagent within the capillary cavity, e.g. an immobilised antigen or antibody, can be significantly less than the whole area of the capillary cavity, and arranged so that all of the sample liquid must pass over the immobilised reagent as liquid is drawn into the cavity. In this way a sample-concentration effect can be achieved, with a resulting surface concentration of relevant analyte material (e.g. complementary antibody or antigen) higher than would result from uniform adsorption over the whole capillary cell area. Such an immobilised coating over a restricted threshold area of the capillary cell surface can form in effect a selective barrier to allow passage of sample liquid beyond the barrier with selective retention of an analyte material of interest.

In the use of devices according to the invention, a drop of sample liquid can be placed on a collection surface of the test device, or else the device can be dipped into a quantity of liquid to be sampled. If ancillary reagents are needed they can be dosed separately, or they can be carried in dry releasable form on a part of the device to be contacted in use by sample liquid, e.g. a surface of the capillary cavity or a surface of a filter if present.

Further variants and particular features of the test devices are described for example below.

According to the present invention we also provide a method of manufacturing specifically-reactive sample-collecting and testing devices, comprising the steps of (a) forming an immobilised specifically-reactive coating on the surface of a sheet material which is to provide part of a multiplicity of the devices, (b) forming

an additional structure which together with said coated sheet material provides for each device of the multiplicity of devices a cavity of capillary dimension for collecting and retaining by capillarity a volume of
5 sample liquid in contact with the specifically-reactive coating, and (c) separating the sheet material into portions each providing one or a plurality of the sample-collecting and testing devices. In this process the specifically-reactive coating can be confluent or
10 continuous or divided into a pattern, e.g. of discrete portions, e.g. as a 2-dimensional array of patches. Where such patches are formed they can be made for example either by first forming a continuous coating and then removing portions of it to leave the desired pattern, e.g.
15 the array of discrete portions, or as a (for example screen-printed or flexographically-printed) array of patches.

The specifically-reactive coating can be a releasable reagent coating, e.g. a coating of releasable antigen or antibody, or derivative thereof, e.g. held in a solid humectant e.g. sucrose glaze, or else for example an immobilised specific binding material such as a covalently bound antigen or antibody or derivative thereof, also
20 possibly coated with a humectant, to form an immunosorbent, with specificity appropriate to a desired assay. The additional structure can for example be a further sheet material bonded to the first sheet material by suitable bonding adhesive, and spaced therefrom by a
25 capillary space, e.g. less than about 1mm, to allow uptake of sample liquid between the sheets by capillarity, preferably in a defined reproducible volume. Either or both sheets may be transparent to light with optically regular, generally smooth, surfaces. The separation of
30 units can be achieved by scribing and breaking the sheet materials, e.g. of glass, siliceous or plastics material,
35

and in the examples described below is carried out so as to leave an external loading surface to which sample liquid can be loaded or applied and from which it can be drawn onto the cavity of the device. The external loading 5 surface has preferably a capacity to contain or hold at least enough liquid (e.g. in the form of a drop of material spread out over the surface) to load the cavity fully.

10 The invention extends to the products of the methods described herein and to the use of those products.

In certain examples of devices according to the invention, the cavity of the device can be a thin planar 15 cavity between two opposite walls forming a cell, and preferably cemented or made into an integral unit. In some cases, for example, the device can include a bonded structure of transparent plates similar to the structure of an unfilled liquid crystal display device as obtained 20 as an intermediate stage in manufacture of liquid crystal displays.

Thus devices according to the invention include those possessing a (for example disposable) translucent or 25 transparent capillary cell, which can be made by the methods described herein, for carrying out specific binding assays, comprising a pair of opposite transparent plates spaced less than about 1mm apart, and sealed together to form an apertured liquid-retentive cell able 30 to take up and retain by capillary action a (preferably defined) volume of (usually aqueous) liquid, and carrying on at least one of its internal surfaces a coating of (preferably immobilised) specific binding agent with a specificity appropriate to the assay to be carried out. 35 "Defined volume" means a volume that is determined substantially by the shape and configuration of the cell

itself and not appreciably by the volume of sample if applied in excess.

- It is important to have an accurately defined 5 (parallel) cavity of capillary gap dimensions in the devices of this invention, but not so important that the total volume taken up by the capillary gap should be defined: the important parameter is rather the volume dosing per unit area of the optical reagent bearing 10 surface of the waveguide, and this is achieved by an accurately defined parallel spacing of the opposite walls of the cavity in the device. The spacing is preferably within the broad range about 0.01 to 1mm, eg. of the order of 0.1 mm, eg. 0.03-0.3 mm, as a compromise between too 15 wide spacings leading to excessive diffusion time of materials in the sample across the gap to the reagent-bearing surface, and too narrow spacings which may collect too little sample.
- 20 Materials suitable to form the cavity are for example glass, eg. sheet soda glass about 1mm thick, silicas and plastics sheet material, eg. acrylic plastics material.
- Where plastics materials are used to form the 25 capillary cells, they can for example be used in the form of precision mouldings, e.g. provided with spacers such as ridges to achieve controlled spacing of the component walls of the capillary cell cavities.
- 30 In some cases the cell has an outer surface portion or lip to which a quantity of sample sufficient to fill the cell can be applied and from which it can easily be made to run into the capillary cell by capillary action. Such a lip can easily be formed by an extension of one of 35 the plates, outwardly beyond the cell aperture, for a distance sufficient to give a surface area large enough

for convenient sample loading. It can also if desired be given a liquid-conductive shape, such as a groove or channel leading towards the capillary cell inlet. An alternative form of inlet is one formed by an aperture in 5 one wall of the capillary cell, e.g. a hole that exposes an area of opposite wall of the cell on to which a sample can be loaded. Selective barriers such as those described elsewhere herein, such as filters and dialysis membranes, can also be located in or adjacent to such 10 inlet apertures, and may be pre-dosed with dry releasable reagents. It is especially convenient to incorporate these features in the plastics capillary cells, and in such cases precision-moulded apertures supplied in a moulded plastics sheet can provide the perpendicular 15 optically flat ends of the capillary cells when the resulting assembly of plural capillary cells is separated into individual cell units.

Preferably the sealing of the cell can be achieved by 20 using a line of epoxy resin, leaving an aperture, e.g. extending the resin along two opposite sides of a rectangular capillary cell, to give a filling aperture and a further aperture left to allow the exit of air from the capillary cell as it fills up. Suitably, the resin can 25 comprise solid particles to ensure a desired spacing for the plates as they bed down on the resin. Particles such as substantially monodisperse ballotini (fine glass particles) of diameter about 100 micron or otherwise corresponding to the chosen capillary gap, or short glass 30 fibre lengths of for example 8 micron diameter and 50-100 micron long (e.g. made by mortar-grinding of long glass fibre and exclusion of long residual fibres by sieving), are suitable to regulate small spacings of the order of the diameter of the ballotini or the fibres. Generally, 35 spacings in the range 5 to 500 microns can be chosen, by way of non-limitative example. Fibres are preferred for

very narrow gaps, as they are more easily obtainable in diameters less than about 50 micron than are monodisperse ballotini: ballotini are preferred for the wider gaps.

- 5 The specific binding agent can be chosen among any of those normally used for purposes of specific binding assay, especially antigens and antibodies. Suitable examples are antoglobulin antibody, or antibody specific to human apolipoprotein A₁ or B, or anti-steroid antibody,
10 e.g. specific for oestrone-3-glucuronide or pregnanediol glucuronide, or non-immunological binding agents such as concanavalin A, or cibacrom blue. They can be immobilised to the glass or silica or plastics surface in any of the ways otherwise practised for such
15 immobilisation. For example, it can be useful simply to coat and dry the binding agent and sucrose on to the carrier surface, either simultaneously or successively. Covalent and other immobilisation can be achieved where desired in any of the ways mentioned in EP specification
20 0 014 530 (Unilever), and references cited therein, especially in the case of plastics materials, and in any of the ways mentioned in "Immobilised Enzymes for Industrial Reactors" (ed. Messing, Academic Press, 1975; especially Filbert, chap 3), or in for example
25 USP 3 652 761 or GB 1 530 997, for a wide variety of carrier materials including siliceous materials such as glass and silica.

- The format of immunoassays, especially e.g.
30 fluoroimmunoassays, which can be carried out in these capillary cell devices corresponds to the known formats of other immunoassays of the prior art. For example, such an assay can be a competitive assay in which an antigen analyte competes for binding sites on a specific
35 immunoabsorbent with a fluorescent competitor, e.g. a fluorescent-labelled antigen analogue. Alternatively,

- the analyte can be contacted with the immunoadsorbent in the absence of the fluorescent ligand and this reaction can be followed by contact between the treated immunoadsorbent and the fluorescent ligand, e.g. as it may
- 5 be released in slow or delayed manner from a releasable coating. In a further alternative, a solution-phase competition assay can be carried out between an analyte and a solution-phase binding partner and fluorescent-labelled competitor, (e.g. soluble antibody
- 10 and competitive fluorescent-labelled antigen), in the presence of an immobilised antiglobulin carried on the capillary cell surface to immobilise the antibody participating in the assay, e.g. after the formation of the mixture of labelled and unlabelled immune complex.
- 15 In a further alternative, a sandwich test or antiglobulin test can be carried out in which the immunosorbent and fluorescent ligand (e.g. both antibody, or in the antiglobulin test respectively an antigen and
- 20 antiglobulin) both have specific affinity for the analyte (the antigen or antibody) under test.
- In a further alternative, a more general ligand-binding reaction may be used, eg. an immobilised
- 25 layer of concanavalin A, which competitively binds glucose and a fluorescently-labelled dextran, or a layer of a dye such as cibacrom blue, which specifically binds fluorescent and unlabelled albumin or other proteins.
- 30 Similarly, luminescent assays may be carried out using labels which participate in chemiluminescent or bioluminescent reactions, eg. luciferase or horseradish peroxidase.

Embodiments of the invention are illustrated for example by the accompanying Figures 1-4 and associated description.

5 Figure 1 shows a diagrammatic section through a disposable capillary cell device according to one embodiment of the invention.

10 Figure 2 shows a diagrammatic plan of the cell device of Figure 1, and includes a line I-I to show the line of section of Figure 1.

15 Figure 3 shows in diagrammatic fragmentary plan an intermediate stage in the manufacture of a plurality of devices as of Figures 1-2.

20 Figure 3a is a schematic fragmentary plan corresponding to a variant on the arrangement shown in Figure 3.

25 Figure 4 shows in schematic perspective view a specifically-reactive capillary cell device according to an embodiment of invention.

30 Figures 5 and 6 show a schematic plan and section of the device of Figure 4.

35 Figures 7 and 8 show a corresponding schematic plan and fragmentary section of an alternative device, a modification of the device of Figures 4-6.

40 Figures 1-2 show a capillary cell device of a size to be handled easily, e.g. about 3cm x 1.5 cm. The device comprises upper transparent (e.g. plastics, glass, or silica) plate 1 and lower transparent (e.g. similar) plate 2 (about 1mm thick) fixed together in parallel opposed and

spaced relation, less than 1mm apart, by bonding tracks 3 of suitable (e.g. epoxy) adhesive to form a capillary cell cavity 4, open at both ends, which communicates with the outside through a first discontinuity in the bonding 3

5 arranged to form a cell aperture at side 5 of plate 1. Another discontinuity is present at the other end of bonding 3, to leave another aperture, to allow exit of air when a sample liquid is loaded into the cell. Plate 2 is larger than plate 1 and has a portion 6 extending away

10 from the aperture. Portion 6 of plate 2 acts as a platform or threshold or lip onto which a drop of sample liquid can be applied, so that this liquid can be made to fill the capillary cell cavity 4 by capillary flow. Cavity 4 attracts and contains a definite and adequately

15 reproducible volume of liquid when loaded in this way.

Immobilised to the inner surface of the capillary cell is a layer 7 of material relevant to the test procedure in which the capillary cell is to be used. In

20 the example shown in the drawings the layer 7 is a patch of material carried on plate 2. For the purpose of immunoassay, it can be for example an area of immobilised immunosorbent sensitisation, e.g. immobilised antibody, relevant to an immunoassay. There can be more than one such layer, e.g. a layer on plate 1 as well as plate 2, or a superimposition and/or side-by-side plurality of layers on either plate. For similar or other purposes, the layer 7 or other layer(s) lining the internal surface(s) of the capillary cell can include an electrically conductive

25 layer or layers, as described in our copending application of even date, derived from UK 8415018, of 13 June 1984, and in such a case conductive external connections can be provided by means of conductive tracks or connectors from the interior of the cell to the exterior of the cell, if

30 desired, passing between bonding layer 3 and the surface of the plates. These can for example be made in a manner

35

known per se and used in the conventional surface fabrication of conductive tracks as often employed in the manufacture of semiconductors and liquid crystal displays, e.g. as referenced below.

5

When the cell is intended for making optical measurements, either plate 1 or plate 2 or both should be transparent or translucent.

10 The section shown as Figure 1 presents plates 1 and 2 spaced apart because the line of section does not extend through the bonding tracks 3.

15 The fabrication of a plurality of cells such as that of Figures 1-2 is illustrated by Figure 3, a fragmentary plan diagram showing an intermediate stage in the manufacture of such cells. A large plate 8 of glass or other material to make plates 2 is cleaned and coated in any appropriate way (eg. as described below) with patches 20 of material 7 of any of the kinds described above as well as tracks of bondable adhesive 3. A second plate, not shown, is then adpressed to plate 8, optionally after forming on it bonding tracks corresponding to track 3, and optionally after forming patches or tracks of any other 25 desired material, and the adhesive is cured. Then the assembly is broken or cut along lines shown as dotted lines 9 in Figure 3, and corresponding lines in the upper plate (not necessarily in registration with lines 9, though). The result is to give cells like the cells shown 30 in Figures 1-2.

Figure 3a shows in diagrammatic plan view an alternative and preferable intermediate stage in the manufacture of capillary cell devices as in for example 35 Figures 1 and 2. The main differences between Figures 3 and 3a, apart from variation in shape and detail of

pattern of the devices to be produced, are that waste edges 31 of the sheet material 8 are provided, and tracks of bondable adhesive 3a are provided in such waste edges 31. The purpose here is to achieve better or more 5 convenient control of the spacing between the upper and lower plates: the bondable adhesive tracks 3 and 3a contain the fibres or microspheres described above to regulate spacing of upper and lower plates and the position of tracks 3a helps to avoid irregularity of 10 spacing at the plate edges.

The arrangements of Figures 3 and 3a have been described in relation to the use of sheet glass as a substrate material: especially but not exclusively where 15 plastics sheet is used, it can be convenient to use other than plain sheet material, e.g. the spacer ridges, inlet apertures and filter arrangements described elsewhere herein can be incorporated as part of such sheets before the capillary cells are assembled.

20 The capillary cell device of Figures 1-2, among other devices made according to this invention, can if desired be provided with any convenient form of handling-piece or holder and for this purpose may be provided with any 25 convenient form of fixed or releasable connexion arrangement to engage with such a holder where this is not formed in one piece with the cell device.

For convenience of optical measurement, it is 30 desirable that one or more edges of plate 1 and/or 2 are made substantially optically smooth and perpendicular to the plane of the plate. The remaining edges may if desired be coated with black paint or other light-absorbing material. Alternatively or additionally, 35 absorbing pigment such as carbon black may be incorporated

in the cement such as epoxy that is used to bond the two plates together.

In use, the device of Figures 1 and 2 can transmit
5 fluorescence from an adsorbed fluorescent material along
either or both of the plates 1 and 2 in the manner of a
waveguide or optical fibre. Transfer of useful light
across the boundary of the plate can occur by the
evanescent wave located very close to the interface. If
10 desired, a thin layer of for example silica or magnesium
fluoride, of the order of a quarter-wavelength thick at
the wavelength of interest, may be fabricated on to the
plate before deposition of the biochemical reagent, in
order to improve light transfer across the boundary. The
15 thickness and refractive index of the dielectric layer are
preferably together chosen to maximise the intensity of
the evanescent wave associated with light transmitted in
the plate in a path that corresponds to total internal
reflection near (and slightly above) the critical angle
20 for the boundary between the plate material and the sample
liquid, e.g. usually in the range about 1° - 5° above the
critical angle. The optimum refractive index of the
dielectric layer is as close as possible to that of the
sample liquid: practically it is most usual to choose a
25 dielectric of magnesium fluoride ($n = 1.38$) or silica
($n = 1.46$) when glass is used. The optimum thickness for
the layer is expressed in terms of the angle of incidence
 P of the light in the plate at the plate-dielectric
interface, and the refractive indices of the media: n_1 of
30 the sample liquid, n_2 of the plate, n_3 of the dielectric,
 L is the wavelength of the light in use:-

$$t = \frac{L}{4} \cdot \frac{\left[1 - \sqrt{\arccos \sqrt{\left(\frac{n_3^2 - n_2^2 \sin^2 P}{n_3^2 - n_1^2} \right)}} \right]}{\sqrt{(n_3^2 - n_2^2 \sin^2 P)}}.$$

In further derived alternative examples, continuous or discontinuous thin metal layers e.g. of silver or indium, preferably overlain by a vacuum-deposited very thin corrosion-resisting layer such as silica, may also 5 be deposited on either or both plates to enable the device to be used in corresponding other methods of optical analysis known in themselves, which exploit such layers, e.g. the per se known phenomenon of surface plasmon resonance, as described in B.Liedberg et al, Sensors and 10 Actuators, 4 (1983) 299-304. In any of these cases the immobilised layers of biochemical reagents may be supplemented or replaced by releasable reagent coatings, e.g. formed by air-drying protein-sucrose mixtures in thin 15 films on the plates: selected and combined according to the particular test chemistry to be performed in the device. The range of chemical or binding reactions that can form part of the tests to be carried out spans the range of known binding-reaction tests, and includes enzyme-linked, fluorescence, luminescence, binding and 20 quenching reactions of any kind based on solid-phase immunoadsorbent or other specific binding adsorbent.

Further details of procedures and material to fabricate cell devices such as the capillary cell device 25 of Figures 1-2 are given in the following Example, further illustrating embodiments of this invention.

Example

30 A sheet of (e.g. soda) glass for example about 1mm thick, and large enough to contain a 2-dimensional array of cell areas, with a plurality of several cell units in each direction, is cleaned by any suitable method, eg. by detergent and ultrasonic treatment and if need be by 35 solvent vapour degreasing in known manner, or by successive hot (80°C) treatments with ammonia hydrogen

peroxide and hydrochloric acid/hydrogen peroxide, water-rinsing and air drying, e.g. at 115°C for 30 minutes. A pattern of patches of a desired protein or other coating is then applied by the following or equivalent technique.

- 5 Covalent coupling of antigen or antibody or other protein is achieved by first reacting the glass with a silane-based coupling compound in known manner. A suitable such reagent is for example, as used here, a terminal amino-alkyl trimethoxysilane, e.g. the
- 10 3-aminopropyl compound, used at a concentration of suitably about 2% v/v in acetone. In alternative methods, another reagent substantially as described in USP 3 652 761 can be used instead. After reaction with the amino-silane reagent, the amino terminals immobilised on
- 15 to the glass are in turn reacted with (e.g. 2% pH 7) glutaraldehyde, excess reagents are removed and the activated glass with immobilised aldehyde groups is reacted with the protein in solution (e.g. 1 mg/ml antibody immunoglobulin), according to component
- 20 techniques well known in themselves. Other proteins can be applied at choice in concentrations of the order of 0.1-1 mg/ml. Treatment at about pH 9.5 for 2 hours at 37°C has been found suitable here. A suitable final active protein loading rate on the glass surface can be
- 25 for example about 0.5 microgram/cm². This is thought to constitute a continuous or near-continuous layer. The dosage or density or specific activity of the immobilised layer is determined by the sensitivity requirements of the particular assay chemistry, which in itself forms no part
- 30 of this invention. Excess reagents can be removed for example washing in strong buffer (0.1M acetate, 0.5M NaCl, pH 4-5), then neutral buffer washing, (pH 7-7.4), followed by pH 9-10 washing and neutralisation, e.g. with neutral tris buffer.

An alternative and sometimes preferred method for coupling protein to glass employs an epoxy-silane reagent, especially the glycidyloxypropyltrimethoxysilane (e.g. 2% v/v in toluene, 70°C for 2½ hours), according to DP Herman et al., J.Chromatogr.Sci., (1981), 19 (9) 470-6. With this reagent the use of aldehyde reagents can be omitted, as the epoxysilylated glass can react directly with protein.

10 It is usually desirable to apply a stabilising coating to such layer-coated surfaces such as a coating of a solid humectant such as sucrose. A suitable example of such a coating is an 8 micron-thick solid sucrose coating applied for example by spin-coating the plate with sucrose solution and drying in air.

15 Releasable coatings can be applied with per se known composition, e.g. carried in admixture with a solid humectant, such as sucrose glaze. We find that it can be 20 desirable to include detergent or inert protein in such coatings (or a soluble salt or buffer material) especially where the active material to be released is itself a protein, and it can be especially desirable to avoid large excess of the reagents in such releasable coatings in 25 relation to the test reactions in which they are to take part.

30 Uniformity of the coating processes can be important, and if appropriate can be checked by test procedures in which radiolabelled and/or fluorescent protein is coated, and then optionally reacted with a further and preferably differently-labelled binding agent. Uniformity of the coating and of its binding capacity can then be checked by surface fluorescent measurement and/or surface 35 radioactivity measurement, eg. using a laboratory gamma-ray scanner.

If it is afterwards desired to etch the coating on the glass, the coated glass is then placed in a confined atmosphere substantially free of moisture or air draughts, e.g. it can be brought close to another flat inert surface

5 to reduce the air gap on the coated side to about 1mm or less. The glass is then illuminated with an ultraviolet patterned image (using preferably light of as narrow as practicable a waveband around c. 280nm) in a pattern corresponding to areas from which the coating is to be

10 etched away, e.g. a grid pattern, to leave a pattern of surviving protein patches. Illumination can for example be carried out using a GE 7-watt mercury lamp spaced a few centimetres from the plate, for a period of about 5-20 minutes. The illumination pattern can be produced by

15 masking close to the plate, or by a real imaging system. The ultraviolet etching used here relies on the same principle as the u.v. etching process described by J A Panitz, I Giaver, in Surface Science, 97 (1980) pp 25-42, to which reference is made.

20

Then a uv-curable epoxy adhesive is printed on to the coated, eg. patch-coated, glass plate in a desired pattern for forming a connexion with an upper spaced plate. The epoxy adhesive is applied by a silk-screen technique which

25 is conventional in itself, and in itself forms no part of this invention.

The epoxy resin can have a small content of short-length glass fibre, about 20 micron in diameter and

30 about 100-200 micron long, (made for example by grinding long glass fibre in a mortar and sieving to remove residual long fibres). A preferred alternative to the glass-fibre pieces is a content of ballotini in the epoxy resin, used as follows. In order to produce a gap of for

35 example 100 micron, correspondingly-sized ballotini are incorporated in the epoxy: a layer of epoxy a little

thicker than the desired spacing between the plates, e.g. 10% thicker, e.g. about 110 micron for a desired spacing of 100 micron, can be laid down by screen-printing, and the addition plate pressed gently into position to spread 5 the epoxy slightly.

If desired, a mirror-image of the first pattern of 10 epoxy adhesive can be applied as a pattern to a second similar sheet of glass, either coated patchwise with the same or a different protein or other coating material, or otherwise uncoated, and the two sheets then brought together, subjected to vacuum or deoxygenation if needed for curing, and cured by ultraviolet illumination. The ultraviolet is applied as an image with a pattern that 15 avoids the patches of coated protein or other material which are to be retained in active form.

After adhesive curing, the two glass plates can be scribed and broken down into individual cell units in any 20 convenient known manner as used in stages in the manufacture of liquid crystal devices, and in particular by the methods referred to in Specifications Nos. CH-627 559, and 629 002, concerning fabrication of liquid crystal display devices. Corresponding steps in the 25 methods of those specifications and of this invention can be performed by similar methods, mutatis mutandis.

A convenient form of cell obtainable by this process comprises two substantially parallel opposed layers of 30 glass, air-spaced by about 5-500 micron, which, together with an incomplete frame of bonding material located between them, (having at least one opening for the inward passage of liquid and possibly also the outward passage of air), form a capillary cell able to take up a defined 35 volume of aqueous liquid. Preferably one of the glass layers extends out beyond the opening of the cell to

enable a drop of liquid to be placed on its surface and pass either wholly or partly into the cell.

Figure 4 shows in schematic perspective a further example of a specifically-reactive capillary cell device according to an embodiment of the invention. The device shown is a disposable single-use test device for carrying out microchemical testing on very small liquid samples. Figure 5 (not to scale) shows a corresponding plan view and Figure 6 a schematic section along lines 6'-6' in Figure 5. The device of Figures 4-6 comprises an upper glass plate 1 and a lower glass plate 2 related as plates 1 and 2 of Figure 1. A reactive layer as 7 in Figure 1 is present on the surface of plate 2 but not shown in the drawings in Figures 4-6. If needed for the purposes of a particular test, an auxiliary reagent can be provided as a releasable coating on the opposite wall of the cell, i.e. the surface of plate 1, so that the reagent dissolves in the sample liquid drawn into the cell. The device of Figures 4-6 can be handled by the user by a handlepiece 42 of a supporting and handling frame indicated generally 41 and made of any convenient plastics moulded material. Arms 43 serve to support the cell assembly and locate it in relation to the operative parts of an optical instrument for optical analysis of the contents of the sample cell. End 44 of plate 2 is optically clear, flat and perpendicular to the plane of plate 2, to allow exit of light arising from the contents of the capillary cell between plates 1 and 2. The other edges of plates 1 and 2 are in this embodiment usefully painted black, and epoxy bonding tracks 3, corresponding to tracks 3 in Figure 1, besides their content of 100 micron ballotini to space plates 1 and 2 apart, also contain carbon black granules to help minimise stray light. A filter paper rectangle 45 is located on a part 46 of plate 2 that extends beyond the length of plate 1 to form a sample-receiving inlet:

the adjacent open end 47 of the capillary gap between plates 1 and 2 is an inlet for the capillary cell delimited by plates 1 and 2 and bonding tracks 3, and filter 45, of a grade sufficiently fine to exclude the 5 passage of red blood cells, is in contact with open inlet end 47 and preferably slightly underlaps plate 2 at end 47. Filter 45 is retained in place if desired by adhesive along parallel lines continuing tracks 3, and can if desired be impregnated with a releasable/auxiliary 10 reagent such as for example a pH buffer salt. Portion 47 of plate 2 extending beyond plate 1 to the optical end 44 of plate 2 can if desired be given a hydrophobic coating.

In use, a sample of liquid to be tested, e.g. a drop 15 of whole blood, can be applied to the inlet zone formed by filter 45, and from such a drop a quantity of relatively cell-free liquid is drawn into the capillary cell. Here take place the binding or other e.g. enzyme reactions appropriate to the nature of the required test and 20 corresponding reagents previously carried in prepared dry form on one or both of the opposite internal cell walls formed by plates 1 and 2, and the loaded cell can then for example be put into a photometric instrument, e.g. that described in copending UK patent application no. 8415019, 25 for optical measurement as described therein.

Figures 7 and 8 illustrate a modification of the device of Figures 4-6. The device has two snap-on handle-and-support pieces, one shown at 41, corresponding 30 to 41 in Figures 46 except that it is detachable, and another handle and support piece 71 comprising handle 72, which is a removable snap-on fit over lateral ribs 73 at the optical end of the device, which comprises similar 35 optical end-faces 44 and 74 of both the spaced-apart plates 1 and 2. Handle 42 is a firmer snap-on fit than handle 72.

The device of Figures 7 and 8 is supplied with handle 72 fitted and handle 42 separated. In this condition a cellulose nitrate or acetate microfine filter or dialysis membrane 75 is exposed at the inlet end of a capillary cell defined as before between plates 1 and 2 and binding tracks 3, but defined here also by a further transverse blackened epoxy bonding track 3a that limits the forward spread of liquid towards ends 44 and 74 of plates 1 and 2, and results in the forward parts of plates 1 and 2 being able to constitute waveguide portions bounded by air on each side. Two lateral gaps are left between tracks 3 and 3a and two corresponding apertures 76 in frame 73 allow for exit of air when the capillary cell fills with sample liquid. Thus, in use, the device can be held by handle 72 and dipped into a source of sample liquid to allow a sample to fill the capillary cell through filter 75. In this embodiment each of plates 1 and 2 carries an immobilised reactive layer 7 and 77 such as an appropriate antibody layer fabricated as described above. If desired, the extent of reactive layers 7 and 77 can be limited to the area shown hatched in Figure 7 on each of plates 1 and 2 between filter 75 and line 78. With such an arrangement, the gently inflowing sample liquid passes over the reactive layers which are each able to abstract their respective ligand, the surface concentration of which can therefore be concentrated in the hatched region.

When the sample has been loaded, separate handle 42 can be snapped-on into place and the more weakly-attached handle 72 can be removed, thereby exposing the optical ends 44 and 74 of plates 1 and 2 for measurement as before, with the modification that two different optical properties may be measured at once by virtue of the separate optical waveguides 1 and 2 and different reactive layers 7 and 77 of the device. A corresponding optical measuring instrument therefore locates a diaphragm in a

position shown by dotted line 79 to separate the respective lights emerging from plates 1 and 2.

5 The invention described herein is susceptible to many modifications and variations within its scope, and in particular extends to the use of any one or more of the singular and several features of the foregoing description and accompanying drawings and their equivalents.

10 Many of the devices made according to the above description can be applied to carry test samples which are to be measured optically by the photometric arrangements and methods described in our copending application derived from UK patent application No. 8415019 (13 June 1984), the 15 disclosure of which is hereby incorporated by reference with the present application.

CLAIMS

1. A specifically-reactive sample-collecting and testing device possessing a cavity or cavities each having a dimension small enough to enable sample liquid to be drawn into the cavity by capillary action, wherein a surface of the cavity carries an immobilised reagent appropriate to the test to be carried out in the device, and wherein said surface is a surface of a transparent solid plate to act as a light-transmissive waveguide and forming a wall of the cavity, said plate having an edge which is substantially optically smooth and transverse to the plane of the plate.
- 15 2. A device according to claim 1, wherein remaining edges of the waveguide are coated with light-absorbing material.
- 20 3. A device according to claim 1 or 2, wherein the surface of the waveguide that carries the immobilised reagent also has a thin layer of material to enhance transfer of light across the boundary of the waveguide by the evanescent wave located close to the interface.
- 25 4. A device according to claim 1, 2 or 3, wherein the immobilised reagent carried on the surface of the waveguide comprises a biochemical specific binding agent, e.g. an antigen or antibody.
- 30 5. A device according to any of claims 1-4, in which a surface of the cavity also carries a releasable reagent coating.
- 35 6. A device according to any of claims 1 to 5, wherein the waveguide comprises glass or plastics material, e.g. soda glass or acrylic plastics.

7. A device according to any of claims 1 to 6,
comprising a bonded structure of transparent plates
cemented in spaced relationship to leave a thin planar
cavity of capillary dimensions between them.

5

8. A device according to any of claims 1 to 7, further
comprising a handling-piece or holder.

9. A method of manufacturing specifically-reactive
10 sample-collecting and testing devices, comprising the
steps of (a) forming an immobilised specifically-reactive
coating on the surface of a transparent sheet material
which is to provide part of a multiplicity of the devices,
(b) forming an additional structure which together with
15 said coated sheet material provides for each device of the
multiplicity of devices a cavity of capillary dimension
for collecting and retaining by capillarity a volume of
sample liquid in contact with the specifically-reactive
coating, and (c) separating the sheet material into
20 portions each providing one or a plurality of the
sample-collecting and test devices.

10. A process according to claim 9, in which the
specifically-reactive coating is divided into a pattern,
25 e.g. of discrete portions, e.g. as a 2-dimensional array
of patches.

11. A process according to claim 10, in which patches
forming the pattern are made by first forming a continuous
30 coating and then removing portions of it to leave the
desired pattern, e.g. the array of discrete portions.

12. A process according to claim 9, in which the specifically reactive coating is a releasable reagent coating, e.g. a coating of releasable antigen or antibody, or derivative thereof, or else an immobilised material such as a covalently bound antigen or antibody or derivative thereof to form an immunoadsorbent.
- 5
13. A process according to claim 9, in which the additional structure is a further sheet material bonded to the first sheet material by a suitable bonding adhesive, and spaced therefrom by a capillary space, e.g. less than about 1mm, to allow uptake of sample liquid between the sheets by capillarity.
- 10
14. A process according to claim 9, wherein the device possesses an external surface to which sample liquid can be loaded or applied and from which it can be drawn into the cavity.
- 15
15. A device according to claim 1, further comprising a means for sample concentration or selective barrier such as a filter or dialysis membrane located in, or in the inlet path to, the capillary cell.
- 20
16. A device according to claim 1, wherein the immobilised reagent overlies a thin coating of conductive material such as a coating of silver up to about 50nm thick, carried on the waveguide surface and capable of showing a surface plasmon resonance effect.
- 25
17. A device according to claim 3, wherein the thin layer material comprises magnesium fluoride or silica.
- 30
18. A device according to claim 1, further comprising an electrode structure for making electrical measurements on the liquid contents of the capillary cell.
- 35

19. A device according to claim 1, wherein the waveguide
is a precision plastics moulding optionally carrying
moulded surface features such as an inlet aperture,
optically smooth moulded light exit edge, or moulded
5 liquid inlet channel.
20. Devices and processes according to claim 1 or 9,
characterised by any one or more of the singular and
several features of the accompanying description and
10 drawings.

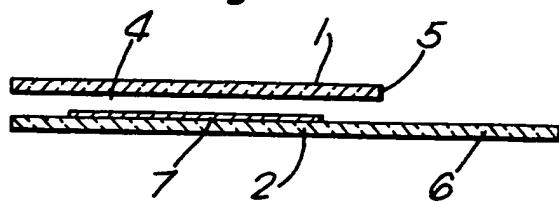
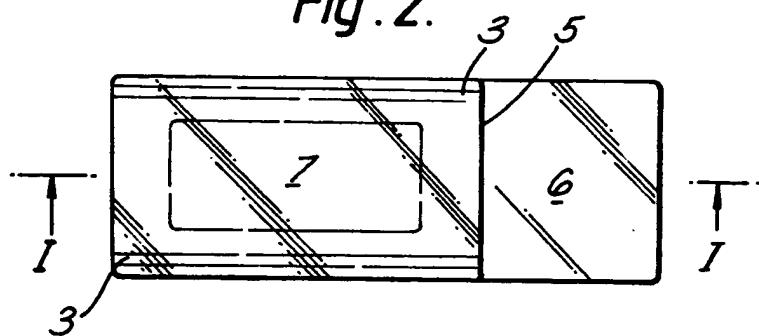
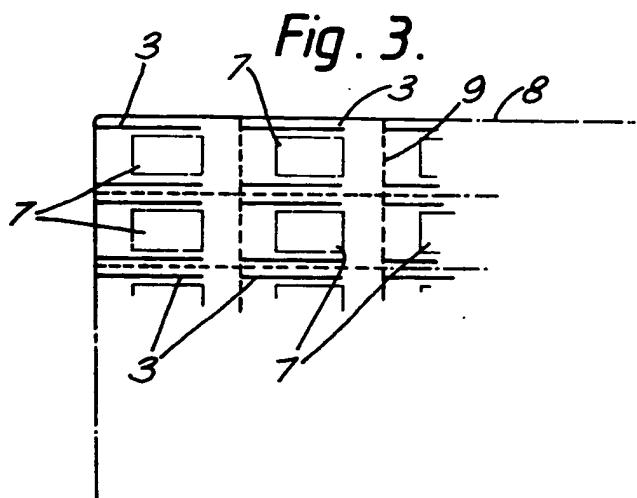
Fig. 1.*Fig. 2.**Fig. 3.*

Fig. 3a.

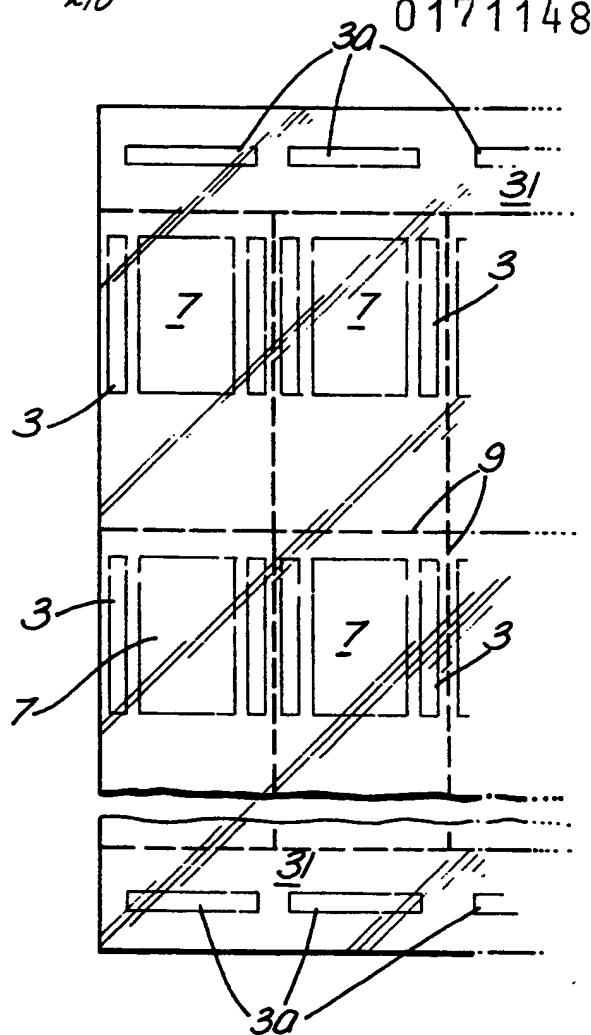


Fig. 4.

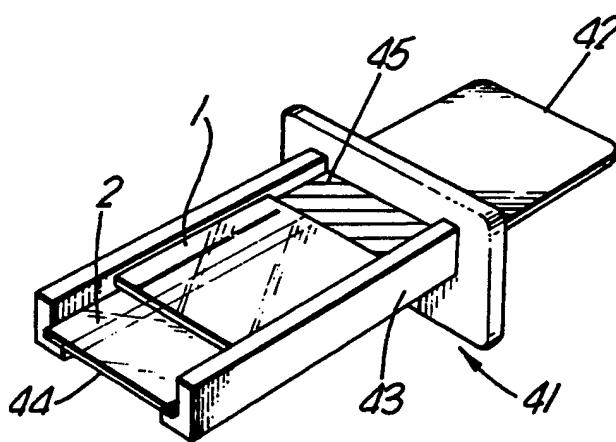
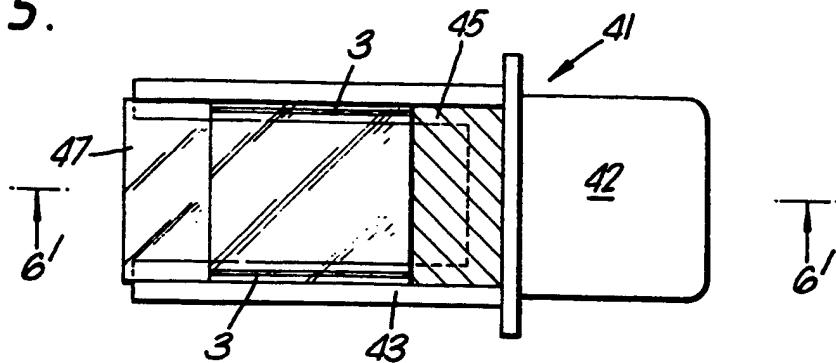
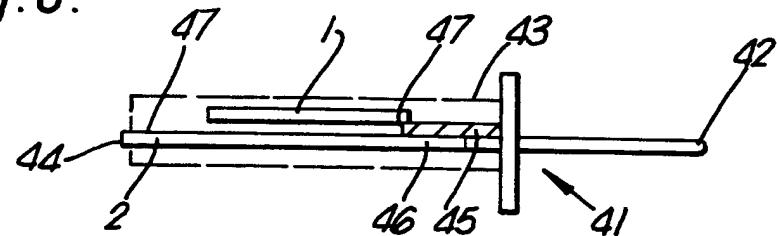
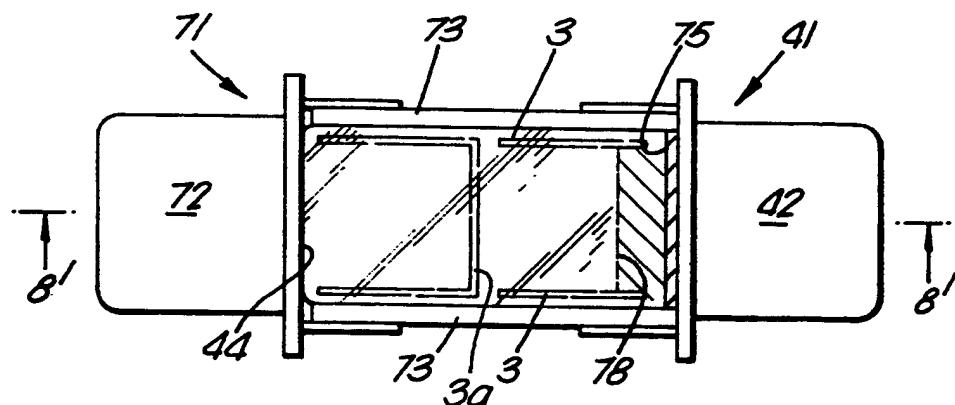
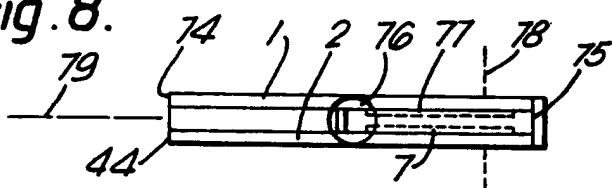


Fig. 5.*Fig. 6.**Fig. 7.**Fig. 8.*



EP 85 30 4169

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)
A,D	EP-A-0 103 426 (M. BLOCK) * Pages 11,13,25 *	1	G 01 N 33/543 G 01 N 21/77
A,D	--- GB-A-2 090 659 (INSTRUMENTATION LABORATORY INC.) * Page 2; figures 1,4,5 *	1,4,7, 8,13, 14	
A,D	--- US-A-4 050 895 (E. HARDY et al.) * Columns 2,7,8; example 3; figures 1,3 *	1,4,6	
A,D	--- EP-A-0 010 456 (EASTMAN KODAK CO.) * Pages 7-9; figures 4,5 *	1,13, 15	
A	--- FR-A-2 325 920 (J. LILJA et al.) * Page 3 *	10,11	
A	--- ANALYTICAL CHEMISTRY, vol. 54, no. 9, August 1982, pages 1071A-1080A, American Chemical Society, US; H. CHABAY: "Optical waveguides" * Pages 1074A,1078A *	1,3,6, 16	G 01 N 33/53 G 01 N 33/543 G 01 N 33/552 G 01 N 21/55 G 01 N 21/77 G 01 N 35/00 B 01 L 3/00
A	--- US-A-4 327 073 (H. HUANG) * Column 8; figure 2 *		
	---	-/-	
The present search report has been drawn up for all claims			
Place of search	Date of compilation of the search	Examiner	
THE HAGUE	20-09-1985	BOEHM CH.E.D.	
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone	T : theory or principle underlying the invention		
Y : particularly relevant if combined with another document of the same category	E : earlier patent document, but published on, or after the filing date		
A : technological background	D : document cited in the application		
O : non-written disclosure	L : document cited for other reasons		
P : intermediate document	& : member of the same patent family, corresponding document		



European Patent
Office

EUROPEAN SEARCH REPORT

0171148

Application number

EP 85 30 4169

DOCUMENTS CONSIDERED TO BE RELEVANT

Page 2

DOCUMENTS CONSIDERED TO BE RELEVANT			Page 2						
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)						
A,D	EP-A-0 034 049 (EASTMAN KODAK CO.) * Pages 7-8 *	1,9,13 ,15							

TECHNICAL FIELDS SEARCHED (Int. Cl.4)									
<p>The present search report has been drawn up for all claims</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 33%;">Place of search</td> <td style="width: 33%;">Date of completion of the search</td> <td style="width: 34%;">Examiner</td> </tr> <tr> <td>THE HAGUE</td> <td>20-09-1985</td> <td>BOEHM CH.E.D.</td> </tr> </table>				Place of search	Date of completion of the search	Examiner	THE HAGUE	20-09-1985	BOEHM CH.E.D.
Place of search	Date of completion of the search	Examiner							
THE HAGUE	20-09-1985	BOEHM CH.E.D.							
CATEGORY OF CITED DOCUMENTS <ul style="list-style-type: none"> X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document 									

ED Form 1603.03.02